PATENT
Attorney Docket No.263365US0X PCT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of
MYLENE WEILL

Serial No.: 10/518,072

Filed: September 22, 2005

Group Art Unit: 1656

Examiner: NASHED, NASHAAT T

For: NOVEL ACETYLCHOLINESTERASE GENE RESPONSIBLE FOR INSECTICIDE RESISTANCE AND APPLICATIONS THEREOF

Declaration pursuant to 37 C.F.R. § 1.132

Hon. Commissioner of Patents and Trademarks
Washington, D.C. 20231

I, Philippe FORT, do hereby declare and state the following:

1. That I received a Ph.D and a post-doctoral degree in Cell Biology. At present I am Head of Department "Cellular Signaling" at CNRS (Centre National de la Recherche Scientifique, France) UMR 5237. Enclosed, please find a copy of my *curriculum vitae* along with a list of references for which I am co-author, which clearly indicates my expertise in the field of Cell Biology.

2. I am one of the co-inventors of the above-captioned patent application and therefore I am very familiar with the subject application. I have read and understood the latest Official Action issued by the U.S. Patent and Trademark Office on February 22, 2010.

3. I would like to point out the following scientific elements regarding the teaching of Bourguet "A" (J. Neurochemistry, 1996, 67, 2115-2123) and Bourguet "B" (Biochemical Genetics, 1996, 34, 351-362).

4. Bourguet "A" and Bourguet "B" show the presence of two types of acetylcholinesterase activities, named AChE1 and AChE2, in *Culex pipiens*. These acetylcholinesterase activities have been identified by carrying out acetylcholinesterase assays using inhibitors, sedimentation and non-denaturing electrophoresis of extracts from *C. pipiens* larvae. The authors have thus identified several bands (protein complexes) of high molecular weight, assumed to be dimers of hypothetical acetylcholinesterase enzymes. Several hypotheses have been made in the Discussion of Bourguet "A" to explain these two AChE activities: the presence of post-translational modifications of a single transcript, interactions with co-factors, or the presence two distinct genes.

5. None of these articles discloses the peptide sequence of the AChE1 enzyme or the nucleotide sequence of the *ace1* gene. Further, these articles do not disclose how to separate the AChE1 enzyme responsible for insecticide resistance from the AChE1 enzyme responsible for insecticide sensitivity.

6. It is interesting to note that in the enclosed article of Bourguet *et al.*, (J Am Mosq Control Assoc. 1998; 14:390-396), published two years after Bourguet "A" and Bourguet "B" by the same authors, the authors refer to only a single *ace* gene. This proves that the hypothesis of two genes encoding two different AChE enzymes was far from being validated.

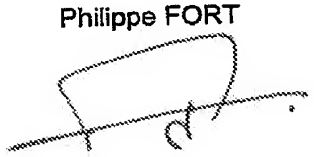
7. The present Invention is based on the formal demonstration of the presence in *Anopheles gambiae* of two genes encoding an acetylcholinesterase: *ace2*, homologous to the single *ace* gene identified in *Drosophila*, and *ace1*, the subject of the present invention.

8. By using cloning and recombinant protein expression methods, the Inventors have shown unexpectedly that *ace1* is associated with resistance to organophosphorous compounds and/or carbamates in *Anopheles gambiae* and *Culex pipiens*. They have further identified the mutation in *ace1* responsible for insecticide resistance, which is identical in both species. The same mutation has since been shown to confer the same type of resistance in other species of mosquitoes.

9. I further declare that all statements made herein of my knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine and imprisonment, or both under section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of this application or any patent issuing thereon.

Date: 15 June 2010

Philippe FORT



FORT Philippe

Personal information

Birth: 11 june 1957
Work address: CRBM, CNRS-UMR5237, 1919 route de Mende F-34293 Montpellier cedex5
tel + 33 467 61 33 56
fax + 33 467 52 15 59
e-mail : philippe.fort@crbm.cnrs.fr

Education

Ingénieur Agronome (INA Paris-Grignon, 1980).
PhD in Molecular Evolution and Paleontology. (Montpellier II University, France, 1982).
Post-doc position on *B. subtilis* sporulation genes. (Oxford University, UK, 1982-84).
Permanent position at CNRS (CR2, 1985; CR1, 1989, DR2, 1997; DR1, 2008).

Scientific carrier

Philippe Fort worked during his PhD on mouse evolution under the supervision of Louis Thaler at ISEM. He then got a post-doctoral position in Joel Mandelstam's lab at Oxford University (UK) to work on a primitive developmental model, the sporulation of *B. subtilis*. In 1985, he took his first position at the French National Center for Scientific Research (CNRS). He ran his own group, first at IGMM in 1990 then at CRBM in 1998, focused on the evolution and the biology of GTPases of the Rho family. His main research interests lay in Rho signalings in epithelial to mesenchymal cell transitions, in particular neural crest cell development and colorectal cancer, and chemical inhibitors of Rho pathways. He is also involved in a long term collaboration on mosquito adaptation with ISEM.

Expertise

Granting Agencies

Member of the steering comitee of the Canceropole GSO (Cell signaling and therapeutic targets).
Member of the scientific comitee of the Ligue Nationale Contre le Cancer (2005-2009).
Member of the Comité Scientifique National du CNRS (2006-2009).
External reviewer for AERES, ECOS-Sud, HFSP, German Research Foundation, MRC, Cancer Research UK, Oncogene and Signal Transduction at University College.

Editorial Board

Editor for "Biology of the Cell".

Ad hoc Journal Reviewer

Cell, Nature Cell Biology, EMBO J., Oncogene, Nucleic Acids Research, Oncogene, Mol. Brain Res., Gene, Biochem. Biophys. Res. Comm., Biology of the Cell, Médecine/Science, FEBS Lett.

Publication record :

95 research articles in journals with reviewing comitee, 32 H-index, over 5,600 cites. 7 published meeting abstracts, 8 reviews and book chapters and 7 patents (2 international extensions).

Major publications :

Bouquier, N., Vignal, E., Charrasse, S., Weill, M., Schmidt, S., Léonetti, J-P., Blangy, A. and Fort, P. (2009). A cell active chemical GEF inhibitor selectively targets the Trio/RhoG/Rac1 signaling pathway. Chem. Biol.16:657-66 *(rated in Faculty of 1000 Biology)*.

Guémar, L., de Santa Barbara, P., Vignal, E., Maurel, B., Fort, P. and S. Faure. (2007) The small GTPase RhoV is an essential regulator of neural crest induction in Xenopus. Dev. Biol. **310**: 113-28.

Boureux, A., Vignal, E., Faure, S. and Fort, P. (2007) Evolution of the Rho family of Ras-like GTPases in eukaryotes. Mol. Biol. Evol. **24**: 203-16.

Duron, O., Fort, P. and Weill, M. (2006) Hypervariable WO prophage sequences describe an unexpected number of *Wolbachia* variants in the mosquito *Culex pipiens*. Proc. R. Soc. Lond. B Biol. Sci. **273**: 495-502 *(rated in Faculty of 1000 Biology)*.

Blangy, A., Bouquier, N., Gauthier-Rouviere, C., Schmidt, S., Debant, A., Leonetti, J. P. and P. Fort. (2006) Identification of TRIO-GEF1 chemical inhibitors using the Yeast Exchange Assay. Biol Cell **98**:511-22 *(rated in Faculty of 1000 Biology)*.

Weill, M., Berthomieu, A., Berticat, C., Lutfalla, G., Negre, V., Pasteur, N., Philips, A., Leonetti, J.P., Fort, P. and Raymond, M. (2004). Insecticide resistance: a silent base prediction. Curr Biol. **14**, R552-3.

Weill, M., Lutfalla, G., Mogensen, K., Chandre, F., Berthomieu, A., Berticat, C., Pasteur,N., Philips, A., Fort, P. and Raymond, M. (2003) The same point mutation in the *ace-1* gene provides high insecticide resistance in the main West Nile and malaria vectors. Nature (London) **423**:136-7.

Pucéat, M., Travo, P., Quinn, M. and Fort, P. (2003) A dual role of the GTPase Rac in cardiac differentiation of stem cells. Mol. Cell. Biol, **14**:2781-92.

Estrach, S., Schmidt, S., Diriong, S., Penna, A., Blangy, A., Fort, P. and Debant, A. (2002) The human RhoGEF Trio is an upstream regulator of the GTPase RhoG in the NGF pathway leading to neurite outgrowth. Curr. Biol., **12** :307-312.

Vignal, E., Blangy, A., Martin, M., Gauthier-Rouvière, C. and P. Fort. (2001) Kinectin is a key effector of RhoG microtubule dependent cellular activity. Mol. Cell Biol. **21**: 8022-34.

Vignal, E., De Toledo, M., Comunale, F., Ladopoulou, A., Gauthier-Rouvière, C., Blangy, A. and P. Fort. (2000) Characterization of TCL, a new GTPase of the rho family related to TC10 and Cdc42. J Biol Chem. **275**:36457-64.

Blangy, A., Vignal, E., Schmidt, S., Debant, A., Gauthier-Rouvière, C. and P. Fort (2000) Trio controls Rac- and Cdc42-dependent cell structures through the direct activation of RhoG. J. Cell Sci. 113: 729-739.

Gauthier-Rouviere, C., Vignal, E., Meriane, M., Roux, P., Montcourier, P.and P. Fort. (1998) RhoG GTPase controls a pathway that independently activates Rac1 and Cdc42Hs. Mol. Biol. Cell. **6**: 1379-1394.

Roux, P., Gauthier-Rouviere, C., Doucet-Brutin, S.and P. Fort. (1997) The small GTPases Cdc42Hs, Rac1 and RhoG delineate Raf-independent pathways that cooperate to transform NIH3T3 cells. Curr. Biol. 7: 629-637.

Vincent, S., Jeanteur, P. and Fort, P. (1992) Growth regulated expression of rhoG, a new member of the ras homolog gene family. Mol. Cell. Biol., **12**: 3138-3148.

Raymond, M., Callaghan, A., Fort, P. and N. Pasteur. (1991) Worldwide migration of amplified insecticide resistance genes in mosquitoes. Nature (London), **350**: 151-153.